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The Spread of Leptospirae in the Body and Antibody Formation in Experimentally Induced Leptospirosis in Irradiated Animals.

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The marked increase of sensitivity of irradiated rabbits, white mice and guinea pigs to infection by the pathogen of Leptospirosis was mentioned in our preceding report. One of the reasons leading to this could be the suppression of antibody production after the irradiation of the animals(Taliaferro).

It is known that the disappearance of the pathogen from the blood of an animal sick with leptospirosis coincides with the appearance of agglutinins and lysins (Varfolomeeva), and is apparently dependent on them, because the immunity mechanism against the given infection is basically connected with the appearance of immune bodies (Aristovskii). Therefore the study of the antibody formation during the course of a leptospiral infection in an irradiated organism and the parallel study of the leptospirae spread in it can explain not only the details of the infection's course under the given conditions, but also several questions of its pathogenesis.

Besides that, the question of the time span in which the infection's pathogen is carried by irradiated animals, and also the question of antibody production in trend with the course of the infection in irradiated animals, have practically not been shown in the literature.

In this work we attempted to resolve, to some degree, the indicated questions, for example , of the leptospiral infection in rabbits, white mice and guinea pigs.

The experiments were conducted on 27 rabbits (1.5-3 kg), 220 white mice (10-15 g) and 20 guinea pigs (200-250 g).

The irradiation of the animals was accomplished with sublethal X-ray doses: for the rabbits 500-600r., for the mice 350 r., and for the guinea pigs 200 r. The mode of irradiation is outlined in a preceding work.

The strain (Rattus ramenka) of the pathogen of leptospirosis used for inoculation was received from the Mechnikov Institute in Moscow. By its antigenic characteristics, this strain is very close to L. canicola and is pathogenic for young guinea pigs weighing approximately 150 g. The causative agent was grown at 25° in double distilled water with 5% rabbit serum. Test cultures containing 80-100 leptospirae in the field of vision were selected for the experiment. The rabbits were intravenously inoculated with 1.5-2. ml of the culture. The mice and guinea pigs were intraperitoneally inoculated with 0.2 ml of the culture. The inoculation adducted the development of a concealed course of infection without lethal results.

The presence of the leptospiremic phase which lasts 4 or 5 days and the subsequent insemination of the organs with a protracted occurence of leptospirae in the kidneys(for several months) is characteristic for leptospirosis (Dal', Varfolomeeva, Nikiforova). In connection with this, the rabbits and guinea pigs were tested periodically for the agglutinin-titer of the blood, and blood samples were taken daily for cultures to detect leptospirae. Mice were killed at various times after their inoculation (2 to 7 mice at a time). Cultures were produced from their blood, and emulsions of the livers and kidneys. The liver and kidney tissues were emulsified with the aid of a special apparatus for the sterile grinding of these organs (Petrov). The cultures survived in a thermostat for more than a month.

In table No 1 are shown the data of the examination of the white mice at various periods after their inoculation which took place 96 hours after irradiation. From table 1 it is apparent that after the inoculation of the normal animals, leptospiremia was observed during the first 96 hours, but after the inoculation of the irradiated animals, it was observed from 7 to 13 days. Leptospirae appeared in the control animals' kidneys during the first five days and then again in the period between the 38th and the 90th day. In the irradiated animals the pathogen appeared during the first 16 days and then again from the 38th through the 190th day. An examination after 220 days showed the presence of leptospirae in the kidneys of both groups of animals.

It is necessary to note that we, as did other investigators (Gorshanova), observed a temporary disappearance of leptospirae from all tissues subjected to examination. However, in the irradiated animals this period was markedly shorter (from the 17th through the 25th day) than in the control animals (from the 6th through the 25th day).

The antibody formation and the simultaneous determination of the duration of the leptospiremic phase in the process of the infection's course were investigated in the rabbits and guinea pigs. The results (Table 2) indicate a suppression of antibody production in the irradiated rabbits. This suppression was very small in the group of animals (Nos. 1, 3, 6 and 10) which were inoculated in the first hours after an irradiation of 600 r. The start of the antibody formation was retarded 24 to 48 hours in the irradiated animals as compared with the control animals. Leptospirae appeared in the blood of the irradiated animals for 6 to 8 days, compared with a 3 to 5 day leptospiremia for the control animals.

Upon the inoculation of the rabbits (Nos 39 through 42) 24 hours after irradiation, a sharp repression of antibody production was observed. The titer of agglutinins in the irradiated animals' blood was 1:40-1:1600 compared with the control groups titers attaining 1:400,000-1:1,600,000. The beginning of the antibody formation was retarded 48 to 72 hours and the leptospiremia lasted 9 to 10 days. An inoculation of rabbits 48 hours post irradiation (600 r.) absolutely did not produce the appearance of antibodies in the blood, and leptospirae appeared in the blood until the very destruction of the animal.

The dynamics of the antibody formation and duration of leptospiremia

in the rabbits of the control and experimental groups are shown on the following chart (See Incl 4).

Experiments on guinea pigs showed the same regularities. For illustration we will take the inoculatory results of two irradiated (200 r.) and two normal animals (Table 3).

By this we clearly see that the suppression and retardation of the antibody production process in experimentally induced leptospirosis in irradiated animals accompany a corresponding prolongation of the time of leptospirae in the blood. This fact, from our point of view, is important not only as one of the moments explaining the more serious course of leptospirosis in irradiated animals. It indicates the specific antibodies' large role in the pathogenesis of leptospirosis, and particularly in the mechanism of freeing an organism from leptospirae.

CONCLUSIONS

1. In the animals inoculated with the pathogen of leptospirosis 2 to 24 hours post irradiation by X-rays, the antibody production was suppressed, but upon inoculation 48 hours after irradiation, the formation of antibodies was completely absent.

2. The leptospiremic phase of the infection lasts longer in the irradiated animals than in the control animals. Thus, the longer the duration of the leptospiremia the mor intensely the antibody formation is repressed.

3. The length of the occurence of leptospirae in the organs of the mice inoculated after irradiation is greater than in the control animals that were only inoculated.

REFERENCES

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Table 1

Detection of Leptospirae in the tissues of white mice at varied times after inoculation.

	24 hour periods after inoculation, / Object of investigation,	1	2	3	4	5	6	7	9	10	11	12	13	14	16	17-25	38	70	90	141	190	220
Inoculation Control.	Blood	x	x	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Liver	x	x	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Kidneys	x	x	x	x	x	-	-	-	-	-	-	-	-	-	-	x	x	x	-	-	x
Irradiation (350 r.) and Inoculation.	Blood	x	x	x	x	x	x	x	-	-	-	x	x	-	-	-	-	-	-	-	-	-
	Liver	x	x	x	x	x	x	x	-	-	-	-	-	x	-	-	-	-	-	-	-	-
	Kidneys	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x	x	x	x	x	x

Designation: x--Growth of leptospirae detected in tissue culture.
- -Tissue culture negative.

Table 2

Inoculatory Results-Rabbits

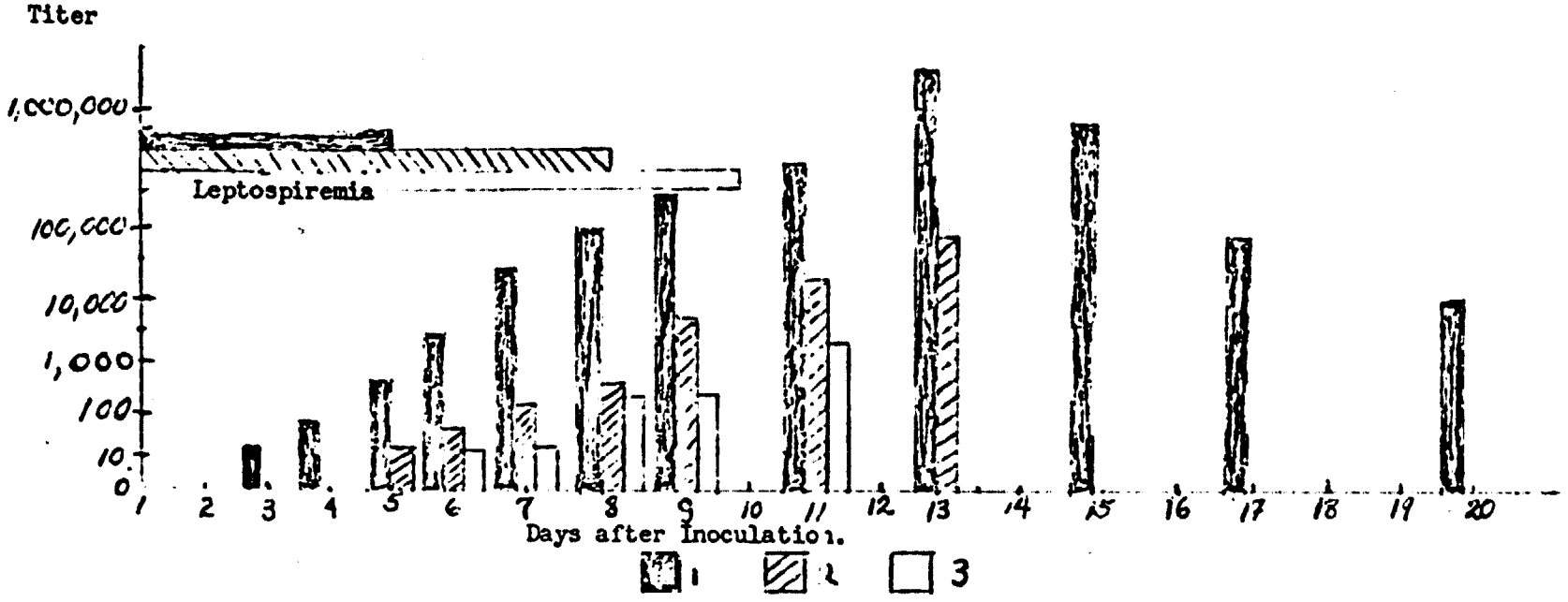
Test	No asgd rabbit.	Weight in grams.	Irradiation dose in roentgens.	No of hours between Irradiation and Inoculation.	Duration of Leptospiremia from moment of inoculation (in days).	Maximum titer of agglutinins.	Day of death from moment of irradiation.
Inoculation Control	2	2620			5	1,620,000	Survived
	5	3170			4	640,000	"
	7	2900			4	800,000	"
	8	2200			4	1,600,000	"
	29	2800			4	800,000	"
	30	2900			3	400,000	"
	43	1610			3	800,000	"
	44	1530			3	1,600,000	"
Inoculation and Irradiation	1	2390	600		8	80,000	13th
	3	2930	600	2-3	7	128,000	8th
	6	2800	600		6	1,280,000	Survived
	10	2440	600		6	1,600,000	13th
	39	1860	500		10	1,600	13th
	40	1530	500	24	10	320	13th
	41	1620	500		10	800	13th
	42	1750	500		9	40	10th
	25	2810	600	48	7	0	9th
	26	2580	600		7	0	9th
			600				
Irradiation Control.	4	2600	600				Survived
	9	2500	600				"
	11	2400	600				9th
	27	2590	600				Survived
	28	2580	600				"
	35	1550	500				"
	36	1590	500				28th
	37	1610	500				Survived
	38	1300	500				

Table 3

Inoculatory Results-Guinea Pigs.

Test	No assigned Guinea Pig.	Weight in grams.	Irradiation dose in Roentgens.	Time between irradiation and inoculation (in hours)	Duration of Leptospiremia (in days).	Agglutination titers in the days after inoculation.					
						4	7	9	11	14	17
Inoculation Control.	13	250			4	50	1,350	4,000	4,000	36,000	36,000
	14	230			4	50	1,350	1,350	1,350	1,350	36,000
Irradiation and Inoculation	5	270	200	48	8	0	50	150	450	Died	
	6	240	200	48	8	0	0	150	Died		

Titer of Antibodies and Duration of Leptospiremia in Rabbits Inoculated with Pathogens of Leptospirosis.



1-Rabbit No 2 (Inoculation Control)

2-Rabbit No 1 (Inoculated three hours after irradiation)

3-Rabbit No 41 (Inoculated 24 hours after irradiation)